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(54) Title: PATHOGENICITY DETERMINANTS WHICH CAN BE USED AS TARGETS FOR DEVELOPING MEANS FOR PREVENTING AND CONTROLLING BACTERIAL INFECTIONS AND/OR SYSTEMIC DISSEMINATION

(57) Abstract: The invention relates to a method for identifying and selecting a gene required for the proliferation *in vivo* of a pathogenic microorganism, comprising :- using a strain of the pathogenic microorganism,- generating mutants for inactivation in the genes encoding these factors,- determining the virulence of these mutants on an expiremental model of infection, and their effect on enteric colonization in an axenic mouse model, and- selecting the bacterial genes essential for resistance to serum in vitro, and essential, in the host, for dissemination in the serum.Application to the screening of compounds which inhibit the products of the genes identified, and to the inhibition *in vitro* of the proliferation of a pathogenic microorganism in serum.

Pathogenicity determinants which can be used as targets for developing means for preventing and controlling bacterial infections and/or systemic dissemination

The invention relates to pathogenicity determinants which can be used as targets for developing means for preventing and controlling bacterial infections and/or systemic dissemination.

Current treatments for infectious diseases of bacterial origin are based on the inhibition of essential bacterial targets *in vitro* using antibiotics. These targets are conserved in many bacterial species and make it possible to treat various types of infection. However, broad-spectrum antibiotics are active on the host's commensal flora, which promotes the acquisition and transfer of mechanisms of resistance to these antibiotics, hence a limiting of the effectiveness of current treatments with antibiotics. A need therefore exists for novel antibacterial treatments.

In this regard, the invention provides a novel strategy, the aim of which is to specifically target pathogenic bacteria without significantly altering their growth at their portal of entry into the host organism, where they are in a situation of commensalism. These pathogens are in particular the bacteria responsible for serious systemic infections, such as *E.coli*, in general *Enterobacteria*, *Pseudomonas*, *Acinetobacter*, *Moraxella* and *Neisseria* and, for the gram positives, the bacteria of the genus *Staphylococcus*, *Enterococcus* and *Streptococcus*.

It is known, specifically, that the bacteria responsible for serious infections are capable of growth in the presence of serum and are resistant to the bactericidal action of

complement. This resistance allows dissemination of the infection, *via* the blood, to the various tissues of the host's body.

The ability of bacteria to grow in human serum is due to different pathogenicity/virulence factors. Among those frequently cited, mention will be made of the physical barrier, represented by the capsule, for access of complement to the bacterial membrane, the sialic acids of the capsule or of the O antigen which promote binding of factor H to c3b, and particular surface proteins such as PorA (*Neisseria gonorrhoeae*), YadA (*Yersinia pestis*) or protein M (*Streptococcus pyogenes*), which bind factor H, all these factors preventing complement activation.

Other proteins expressed or bound by the pathogens have proved to be important for resistance to complement and cause cleavage of complement factors or inhibit their binding to the surface of the bacterium (Rautemaa R.; Meri S., Microbes and Infection 1999, 1:785:794).

The lipopolysaccharide (LPS) of gram-negative bacteria is known to be a virulence factor, but the role of its various constituents on the resistance to serum has not been established for all bacterial species. For example, in some studies in *E.coli*, the O antigen is considered to be determinant (Burns S.M. Hull S.I. Infect Immun, 1998, Sept 66(9):4244-53); in other studies, the O antigen is thought to be less determinant than the capsular antigens for resistance to serum (Russo T. et al., Infect Immun, 1995, Apr. 63(4):1263-9). Furthermore, the importance of these factors on intestinal colonization is unknown.

The inventors have carried out a systematic analysis of mutants for inactivation of the genes required for surface polysaccharide synthesis, and have demonstrated, in *Escherichia coli* strains responsible for extra-intestinal infections, EXPEC, which genes are essential for the resistance to serum and the dissemination in the blood. These results are based on the study of the effect of mutations on virulence and intestinal colonization in an animal model.

The invention is therefore directed towards a novel methodology for defining the targets required for virulence, and not essential *in vitro*, and thus providing novel anti-infectious agents specific for pathogenic bacteria, in particular for extra-intestinal *E.coli*, responsible for severe infections, as well as Gram positive strains, such as *Streptococcus agalactiae*. It is also directed towards the products of the genes required for resistance in the serum and virulence *in vivo*.

The method of the invention for identifying and selecting a gene required for the proliferation *in vivo* of a pathogenic microorganism is characterized in that it comprises:

- using a strain of the pathogenic microorganism,
- generating mutants for inactivation in the genes encoding the virulence factors,
- determining the virulence of these mutants on an experimental model of infection and their effect on enteric colonization in an axenic mouse model, and
- selecting the bacterial genes essential for resistance to serum *in vitro* and essential, in the host, for dissemination in the blood.

The pathogenic microorganism is in particular an EXPEC strain of *E.coli* or a *Streptococcus agalactiae* strain.

The virulence gene inactivation mutants used in this method fall within the scope of the invention.

Said mutants are characterized by the following properties : they are sensitive to serum; they are avirulent in mice model and they are able to colonize gut of axenic mice.

The invention is also directed towards the pathogenicity or virulence factors encoded by nucleic acids thus identified, which are necessary for the dissemination *via* the blood, but do not significantly affect the intestinal or mucosal colonization of pathogenic bacteria such as *E.coli, Salmonella typhimurium, Klebsiella pneumoniae, Yersinia pestis, Serratia marcescens, Haemophilus influenzae, Pasteurella multocida, Vibrio cholerae, Pseudomonas aeruginosa, Acetinobacter, Moraxella catarrhalis, Burkholderia pseudomallei, Neisseria meningitidis, Neisseria gonorrhoeae, Campylobacter jejuni, Helicobacter pylori, Bacteroides fragilis, Clostridium acetobutylicum, Mycobacterium tuberculosis, Streptococcus pyogenes, Streptococcus agalactiae, Staphyloccus aureus* and *Enterococcus*.

The invention is in particular directed towards the pathogenicity or virulence targets encoded by isolated or purified nucleic acids having sequences SEQ ID Nos 16-30.

The pathogenicity or virulence targets of the invention are more particularly encoded by nucleic acids having sequences SEQ ID Nos 16,17,19-30.
Said nucleic acids are cDNAs or RNAs.

It particularly relates to pathogenicity or virulence targets encoded by nucleic acids of *E.coli*.

In another embodiment of the invention, the pathogenicity or virulence targets are encoded by nucleic acids of *Streptococcus agalactiae*.

The invention is also directed towards the vectors comprising at least a nucleic acid coding for a pathologenicity or virulence target such as above defined and also the host cells containing at least one vector under the control of a suitable promoter.

The invention is also directed towards pathogenicity or virulence factors corresponding to isolated or purified polypeptides or peptides having one of the amino acid sequences SEQ ID Nos 1-15.

It more particularly relates to pathogenicity or virulence factors corresponding to isolated or purified polypeptides or peptides having the amino acid sequences SEQ ID Nos 1,2,4-15.

The antibodies which are capable of binding specifically to the peptides and polypeptides corresponding to said factors are also part of the invention.

These nucleic acids and peptides or polypeptides constitute targets for identifying compounds with a specific inhibitory effect on the systemic dissemination of a bacterial infection, and not on mucosal colonization or, for enterobacteria, on intestinal colonization, which makes it possible to preserve the commensal flora and to avoid the selection of resistance to the compounds.

The invention is thus directed towards the method for inhibiting the proliferation of a pathogenic microorganism in serum, comprising the use of an effective amount of a compound capable of inhibiting the activity, or of reducing the amount, of a nucleic acid as defined above, or of a compound capable of inhibiting the activity of a polypeptide or of a peptide as defined above.

It is also directed towards a method for screening compounds capable of inhibiting the expression of these nucleic acids or of the corresponding polypeptides and peptides, comprising bringing them into contact with the test compound, demonstrating the possible effect of the compound on their activity, and selecting the active compounds.

It is also directed towards a method for screening compounds capable of inhibiting the biochemical and/or enzyme activity of the polypeptides and peptides expressed by said nucleic acids.

The compounds thus selected are used, in accordance with the invention, to produce medicinal products for inhibiting a bacterial infection, in particular an extra-intestinal infection in the case of enterobacteria.

The invention thus provides a novel strategy and novel means for preventing or treating systemic bacterial dissemination, bacteraemia and septicaemia.

Other characteristics and advantages of the invention will be given in the following examples, with reference to Figures 1 to 3 and tables 1 to 5, said figures representing, respectively,

- Figure 1, the growth of S26 and of the mutant pg23 in serum,
- Figure 2, the growth of S26 and of the mutant pg23 in decomplemented serum, and
- Figure 3, the virulence of the DltD mutant of *S.agalactiae*.

Example 1 : gene corresponding to SEQ ID N°23:

1- Inactivation of the gene of interest

The general strategy, based on a recombination system, consists in interrupting a gene, by allelic recombination, with a gene for selection (a gene for resistance to antibiotic in the present case) carried by a linear DNA fragment.

Initially, a plasmid is introduced into the bacterium (for example *E.coli*), so as to introduce, in trans, the proteins which will induce the recombination. The plasmid carrying an ampicillin resistance gene is thermosensitive (30°C), which will make it possible to easily eliminate it after use in the bacterium.

The plasmid is introduced into the bacterium by electroporation. After electroporation, the ampicillin-resistant bacteria will be those which have integrated the plasmid, and will be selected. This step is entirely carried out at 30°C, the permissive temperature for the plasmid.

Synthesis of the PCR fragment specific for the target gene (*pg23*)

A PCR is carried out, on a matrix plasmid carrying the selection gene (chloramphenicol resistance), using primers pg23P1 and pg23P2 of sequences SEQ ID No 31 and SEQ ID No 32, respectively, made up of two parts:

in 3′: 20 bp homologous to the selection gene (chloramphenicol resistance): P1 or P2

in 5′: 40 bp homologous to the target gene (*pg23*): H1 or H2

pg23P1: 5'

TCGTGCAGGCCAACCTGCACAACAGAGTGATTTGATTAACGTGTAGGCTGGAGCTGCTTC

3'

H1 P1

Pg23P2: 5'

CAGGGTGCTGGCGCTCACCATTTCCGGAGACAGCTTAGACACATATGAATATCCTCCTTA

3'

H2 P2

A DNA fragment consisting of the selection gene (CAT: Chloramphenicol Acetyl Transferase) flanked by the regions homologous to the target gene H1 and H2 is thus obtained.

Step for inactivation of the target gene

The bacterium containing the plasmid is cultured in LB medium at 30°C with shaking, in the presence of 100 mM ampicillin and of 1 mM L-arabinose so as to induce the recombination system. When the bacteria are in the exponential growth phase (OD_{600nm}=0.5), the culture is stopped, and the bacteria are harvested and made electrocompetent. The PCR product specific for the target gene (*pg23*) is introduced into the bacterium by electroporation. The bacteria are then cultured in a non-selective rich medium (SOC medium) at 37°C with shaking for 2 hours, and then plated out onto selective LB agar medium. After 18 hours at 37°C, only the bacteria which have integrated the gene for resistance to the antibiotic will have grown.

Verification of the insertion of the resistance cassette

In order to verify the insertion of the resistance cassette, PCR reactions are carried out directly using colonies. Three

pairs of primers are used: a pair in which the primers FR1 and FR2 frame the target gene, and two pairs using a primer located inside the resistance cassette, the other primer being located either upstream or downstream of the target gene.

Isolation of the mutated bacteria and elimination of the plasmid

The colonies thus verified by PCR are successively re-isolated on selected medium, twice on non-selective medium and a final time on selective medium at 37°C. Finally, the selected bacteria are tested for sensitivity to ampicillin, which reflects the absence of the plasmid. Three clones are thus chosen for each type of mutant.

2 - Test for the mutant with respect to resistance to the bactericidal activity of serum

The serum used is of human origin. In each experiment, growth was also effected for the wild-type strain (S26, clinical isolate of *E.coli* particularly resistant to serum and virulent in mice) and a strain, ECOR4, lacking a capsule and lipopolysaccharide (LPS). The growths were effected in triplicate and in two different sera. The growths were effected in parallel in complemented and decomplemented (30 min at 56°C) serum in order to verify that the effect observed was due only to the lytic action of complement.

Using a preculture of two hours in RPMI reference minimum medium, the bacteria are brought into contact with 100% serum, at a starting inoculum of 10^4cfu/ml. Counts are then performed at times 0, 1 and 4 hours, by plating various dilutions out on LB agar medium in the presence or absence of antibiotic. After 18 hours at 37°C, the bacteria are counted and a growth curve

is produced from the results obtained. These results are given in Figures 1 and 2.

In this example, the mutant Δpg23 exhibits considerable sensitivity to the serum: a difference from the wild-type strain of more than 2 log at 1 hour and of more than 4 log at 4 hours is in fact observed. In addition, the results obtained in decomplemented serum and with the strain ECOR4 in serum indicate that the effect observed is indeed due to the bactericidal action of complement.

3 - Study of the virulence in a mouse animal model

Preparation of the inoculum

The wild-type mutated bacteria are isolated from the strain, stored at -80°C, on an LB agar dish with or without antibiotic, and incubated at 37°C for 18 hours. A preculture is prepared in liquid medium. Using a 1/10th dilution in 10 ml of LB, the culture is regrown at 37°C with shaking for 2 hours. After culturing for 2 hours, the OD_{600nm} is measured and various dilutions are prepared in physiological saline, so as to obtain the desired inoculum. For the wild-type strain S26, the LD_{50} corresponds to an inoculum of 5×10^5 cfu/mouse and the LD_{100} corresponds to an inoculum of 1×10^6 cfu/mouse.

Virulence test

The mice (6-week-old Balb/c) are given an intraperitoneal injection and the bacterial solution injected represents a volume of 100 µl. Five mice are used per dose. For S26Δpg23, 4 inoculums were tested and the survival rate was measured after 24 and 48 hours post-injection. In each experiment, the study was carried out in parallel with the wild-type strain, the LD_{50} of which is 5×10^5 cfu/mouse.

The mutant S26Δpg23, injected at a dose equal to 10 times the LD_{100}, causes no mortality, the mutation of the *pg23* gene in the *E.coli* strain K1 S26 is therefore responsible for a considerable decrease in the virulence.

4 - Study of the intestinal colonization in an axenic mouse animal model

The entire experiment is carried out in a sterile environment, with sterile instruments, in an isolator, and the mice are given sterile food.

Mice

These are 6- to 8-week-old axenic female mice of the C3H/He J line.

Four animals are used per bacterial strain.

Preparation of the inoculum

The wild-type and mutated bacteria are isolated from the strain, stored at -80°C, on an LB agar dish with or without antibiotic, and incubated at 37°C for 18 hours. After culturing the strain in liquid medium, various dilutions are prepared in physiological saline, so as to obtain an inoculum of 10^7 cfu/ml.

Colonization test

The bacterial inoculation is carried out orally. During the 24 hours preceding inoculation, the mice are deprived of water. They are then given a bacterial solution at 10^7 cfu/ml to drink for 4 hours. The volume of drink is measured at 0 and 4 hours, and, on average, a mouse absorbs 5 ml of this bacterial solution. The faeces are then sampled at various times, and a

bacterial count is performed, taking the faeces up in physiological saline and plating out various dilutions on an LB agar dish with and without antibiotic.

The results are given in table 1 herein below.

TABLE 1

	CFU/mg faeces	
Time in hours	S26wt	S26Δpg23
0	0	0
4	6.85E+05	1.65E+05
25	1.86E+06	2.84E+06
118	8.34E+06	7.94E+06
456	4.14E+06	6.64E+06

For the wild-type strain S26, as well as for the mutant S26Δpg23, colonization in the intestine was stably established. No difference is observed between the wild-type strain and the mutant Δpg23. The colonization is confirmed on the final day by removing the intestine and counting the bacteria after grinding of this organ.

5 - Cloning and expression of the selected polypeptide

The nucleic acid encoding the polypeptide is cloned into a prokaryotic expression vector such as pET-14b with an N-terminal poly-his tag, according to conventional cloning methods.

The recombinant plasmid is then used to transform the *E.coli* strain BL21. The transformed cells are selected in the presence of ampicillin and the colonies are isolated. They are then cultured in the presence of IPTG in order to induce expression of the protein. The clones producing the protein

are cultured and the total proteins are extracted by cell lysis. The recombinant protein is purified with a histidine tag affinity column, according to the manufacturer's protocol.

The protein thus obtained is purified and used *in vitro* to measure its enzyme activity.

Example 2 : serum sensitivity and LD_{50} determination of mutant strains in the mice model of infection

Said mutants were also compared to the wild type S26 *E.coli* strain for LD_{50} determination in the mice model of infection.
As presented in Table 2 below, the number of colony forming unit (cfu) counted after culture for four hours in serum was higher in the wild type (wt) S26 strain than in mutants indicating that mutants were sensitive to serum killing.
All the different mutants were either much less virulent in mice than the wild type strain as shown by the increase in LD_{50} (lethal dose 50), or completely avirulent as no dose killing 50% of mice could be reach with the mutants.

Table 2

Serum sensitivity and virulence attenuation for *E. coli* K1 S26 mutants in the proteins corresponding to sequence number 1 to 13

Sequence Number	Serum sensitivity #Δlog (cfu/ml serum)	Virulence attenuation * Δlog (LD50)
1	+4	avirulent[a]
2	+4	+1
3	+5	+1
4	+4	+1
5	+4	+2,5
6	+4	+0,5
7	+4	+0,5
8	+4	avirulent[a]
9	+1	avirulent[a]
10	+2	avirulent[a]
11	+4	+2
12	+4	+2
13	+4	avirulent[a]

avirulent[a]: no dose killing 50% of mice could be reach with that mutant.

Δlog (cfu/ml serum) = log (cfu S26wt / ml serum) - log (cfu S26 mutant / ml serum)
values obtained after 4 hours in serum

* Δlog (LD_{50}) = log (LD_{50} S26mutant) - log (LD_{50} S26wt)
values obtained 48 hours after inoculation

The mutants of genes encoding the target proteins corresponding to sequences 1 to 13, which were attenuated for virulence, were still able to colonize the intestine of axenic mice as shown by persistence of bacteria in the faeces of the

animals over a period of eight days. These results are presented in Table 3.

Table 3

Gut colonization for *E. coli* K1 S26 wt and mutants in the proteins corresponding to sequence number 1 to 13 in an axenic mouse model

	Sequence number	Gut colonization cfu/mg faeces	
		Day 1	Day 8
S26 wt.		* $1,34.10^6$	* $5,29.10^6$
S26 mutants	1	$9,73.10^5$	$2,51.10^6$
	2	$1,02.10^6$	$6,85.10^6$
	3	$1,44.10^6$	$3,48.10^6$
	4	$1,24.10^6$	$1,65.10^6$
	5	$1,15.10^5$	$4,64.10^5$
	6	$9,96.10^5$	$3,51.10^6$
	7	$2,40.10^4$	$2,51.10^6$
	8	$2,84.10^6$	$6,64.10^6$
	9	$1,80.10^6$	$1,51.10^6$
	10	$9,62.10^5$	$2,24.10^6$
	11	$2,72.10^5$	$8,56.10^5$
	12	$3,13.10^5$	$9,09.10^5$
	13	$5,91.10^5$	$1,67.10^6$

* mean values based upon six experiments

The bacteria colonizing the intestine of axenic mice after eight days were characterized to verify that they correspond to the mutant strains that were inoculated orally.

The bacteria recovered from the faeces of animals had a phenotype of chloramphenicol resistance and serum sensitivity, the chloramphenicol acetyl transferase gene inserted during the mutagenesis could also be detected by PCR.

Mutations in genes encoding target proteins (sequence number 1 to 13) were still present in bacteria colonizing the intestine of axenic mice as shown in Table 4.

Table 4

Characterization of bacteria recovered from axenic mice after intestinal colonization by mutants in genes encoding proteins sequence 1 to 13

Sequence Number	Serum sensitivity # ΔLog (cfu/ml serum)	* Mutant genotype
1	+5	Cm^R, PCR +
2	+4	Cm^R
3	+5	Cm^R
4	+3	Cm^R
5	+5	Cm^R, PCR +
6	+2	Cm^R
7	+2	Cm^R
8	Nd	Cm^R
9	+2	Cm^R
10	+3	Cm^R
11	+5	Cm^R, PCR +
12	+4	Cm^R, PCR +
13	+4	Cm^R, PCR +

ΔLog (cfu/ml serum) = log (cfu S26wt / ml serum) - log (cfu S26mutant / ml serum)
values obtained after 4 hours in serum

*The presence of the gene encoding the chloramphenicol acetyltransferase, inactivating the genes encoding the proteins of sequence number 1 to 13, has been verified by PCR and chloramphenicol resistance (Cm^R).

In conclusion, the results presented in this example demonstrate that genes encoding the enzymes involved in the LPS inner core metabolism are not essential in *E.coli* strains

for colonization, but are necessary for resistance to complement and virulence *in vivo*.

They represent as such good targets for inhibitors that will selectively block bacterial replication in blood tissue.

Example 2: mutants of protein SEQ ID N°14

The present invention relates to novel mutant strain of Group B Streptococcus (GBS) (*Streptococcus agalactiae*). In this particular example, the identified targets correspond to gene sequence number 29 encoding a protein sequence number 14 involved in incorporation of D-alanine residues into the cell wall-associated lipoteichoic acids (LTAs) in Gram + bacteria.

The gene sequence number 29 is homologous to the *dltD* gene found in other gram positive bacteria and is the last gene of the dlt operon.

The Gram + bacterial model used is the pathogenic strain *S. agalactiae* NEM316. *S. agalactiae* is a bacterium commonly found in the human flora and is phylogeneticaly close to Gram + bacteria responsible for nosocomial septicemia.

The virulence of GBS mutants in the dlt operon is strongly impaired in mouse and newborn rat models.

Interestingly, the loss of virulence is presumably due to an increased sensitivity to antimicrobial cationic peptides, such as defensins, which are produced by numerous cells types in particular phagocytes.

The use of mutant of *S. agalactiae,* in which the *dltD* gene have been inactivated, demonstrates that the product of that gene is a good target for the development of inhibitors of virulence of *S. agalactiae* as well as against other Gram + pathogens.

Construction of a DtlD mutant in wild type *S. agalactiae* NEM316:

A mutant in the *dltD* gene was constructed from *S. agalactiae* NEM316 strain by inserting, using double cross-over, a kanamycin resistance cassette.

To construct DltD mutant of S. agalactiae NEM316, a promoterless and terminatorless kanamycin resistance cassette aphA-3 within DNA segment internal to dltD were inserted in the same direction of transcription. This was done by ligation after digestion with appropriate enzymes, of PCR products obtained by using the primers of SEQ ID N° 33 and 34 respectively,

SEQ ID N°33 : 5'-CAGTGAATTCGCGTTGACGAAGGCAGG-3', and
SEQ ID N°34 : 5'-GACGGGTACCATACCTATCGTAGGTTG-3', and
the primers of SEQ ID N° 35 and SEQ ID N°36, respectively,
SEQ ID N°35 : 5'-AGTGGATCCACTACACAGGGCTTGATC-3', and
SEQ ID N°36 : 5'-GACCTGCAGCCCTTGATTATCCCTATCC-3'.

A 0.4 kb dltD EcoRI-KpnI fragment was inserted into the thermosensitive shuttle vector pG+host5ΩaphA-3 (Biswas et al., 1993, J Bacteriol. 175:3628-3635) containing the kanamycin resistance cassette to generate pG1Ω EKaphA-3. A 0.8 kb closely spaced dltD region BamHI-PstI fragment was inserted into pG1Ω EKaphA-3 to generate pG1Ω EKaphA-3BP. The resulting vector was introduced by electroporation into NEM316. Transformants were selected on Todd-Hewitt (TH) agar plates containing 10 mg l^{-1} erythromycin at 30°C. Allelic exchange was obtained at the non-permissive temperature (42°C) by homologous recombination using a two-step procedure described previously (Biswas et al., 1993).

A double-crossover event between the homologous sequences resulted in nucleotides deletion and insertion of the kanamycine cassette. Recombinant bacteria containing this insertion deletion were selected for kanamycine resistance.

This chromosome disruption in the dltD gene was confirmed in one of the recombinant clones by sequencing the nucleotides of the mutant.

Sensitivity of the wild type *S. agalactiae* strain NEM316 and the DltD mutant to various antimicrobial peptides :

The sensitivity of wild type *S. agalactiae* NEM316 and DltD mutant to cationic antimicrobial peptides was measured by using a disk diffusion methods. The 2 strains were grown on blood agar plates and incubated for 18 hours at 37°C. Each strain was tested using colistin (50 µg) and polymixin (10 µg) disks. Sensitivity or resistance of NEM316 strain and the DltD mutant to each compound was determined by the size of the growth inhibition area around disk.

The DltD mutant exhibited an increased sensitivity to the cationic antimicrobial peptides colistin, and polymyxin B as shown in table 5.

Table 5

Results of sensitivity to colistin and polymixin B of control strains *S. agalactiae* NEM316 and DltD mutant

	Disc content (µg)	Inhibition area (mm)	
		NEM316	Mutant DltD
Colistin	50	0	14
Polymixin B	10	0	14

Study of virulence in a mouse animal model

We studied the role of DltD in the virulence of *S. agalactiae*. Groups of ten mice (six week-old Balb/c) were inoculated intravenously with 5 x 10^7 bacteria. At 2 days post infection, 80% of mice infected with the wild type strain NEM316 died and

only two deaths were recorded for mice infected with the DltD mutant. Figure 1 illustrates the results obtained with the DltD defective GBS mutant. The result demonstrates that the product of the dltD gene is necessary for virulence of GBS in mice.

CLAIMS

1. Method for identifying and selecting a gene required for the proliferation *in vivo* of a pathogenic microorganism, comprising :

- using a strain of the pathogenic microorganism,
- generating mutants for inactivation in the genes encoding these factors,
- determining the virulence of these mutants on an experimental model of infection, and their effect on enteric colonization in an axenic mouse model, and
- selecting the bacterial genes essential for resistance to serum *in vitro*, and essential, in the host, for dissemination in the serum.

2. Method according to Claim 1, characterized by the use of an *E.coli* strain EXPEC or a *Streptococcus agalactiae* strain.

3. Mutant nucleic acids for inactivation of the virulence genes as implemented in the method according to Claim 1 or 2.

4. Mutant nucleic acids which are sensitive to serum; avirulent in mice model and able to colonize gut of axenic mice.

5. Pathogenicity or virulence targets encoded by isolated or purified nucleic acids corresponding to one of the nucleotide sequences SEQ ID Nos 16-30.

6. Pathogenicity or virulence targets according to claim 5, wherein said nucleic acids correspond to one of the nucleotide sequences SEQ ID Nos 16,17,19-30.

7. Pathogenicity or virulence targets according to claim 5 or 6, wherein said nucleic acids are cDNAs.

8. Pathogenicity or virulence targets according to claim 5 or 6, wherein said nucleic acids are RNAs.

9. Pathogenicity or virulence targets according to any one of claims 6 to 8, wherein said nuclesic acids correspond to the nucleic acids of pathogenic organisms comprising *Escherichia coli, Salmonella typhimurium, Klebsiella pneumoniae, Yersinia pestis, Serratia marcescens, Haemophilus influenzae, Pasteurella multocida, Vibrio cholerae, Pseudomonas aeruginosa, Acetinobacter, Moraxella catarrhalis, Burkholderia pseudomallei, Neisseria meningitidis, Neisseria gonorrhoeae, Campylobacter jejuni, Helicobacter pylori, Bacteroides fragilis, Clostridium acetobutylicum, Mycobacterium tuberculosis, Streptococcus pyogenes, Streptococcus agalactiae, Staphyloccus aureus* and *Enterococcus*.

10. Pathogenicity or virulence targets according to claim 9 corresponding to nucleic acids of *E.coli* or *Streptococcus agalactiae*.

11. Vectors comprising at least one pathogenicity or virulence target according to any one of claims 5 to 10.

12. Host cells containing at least one vector according to Claim 11.

13. Products of expression of the pathogenicity or virulence targets according to any one of claims 5 to 10.

14. Isolated or purified peptides characterized in that they correspond to one of the amino acid sequences SEQ ID Nos. 1 to 15.

15. Isolated or purified peptides according to claim 14 characterized in that they correspond to one of the amino acid sequences SEQ ID Nos 1,2,4-15.

16. Antibodies capable of binding specifically to the peptides according to any one of Claims 13 to 15.

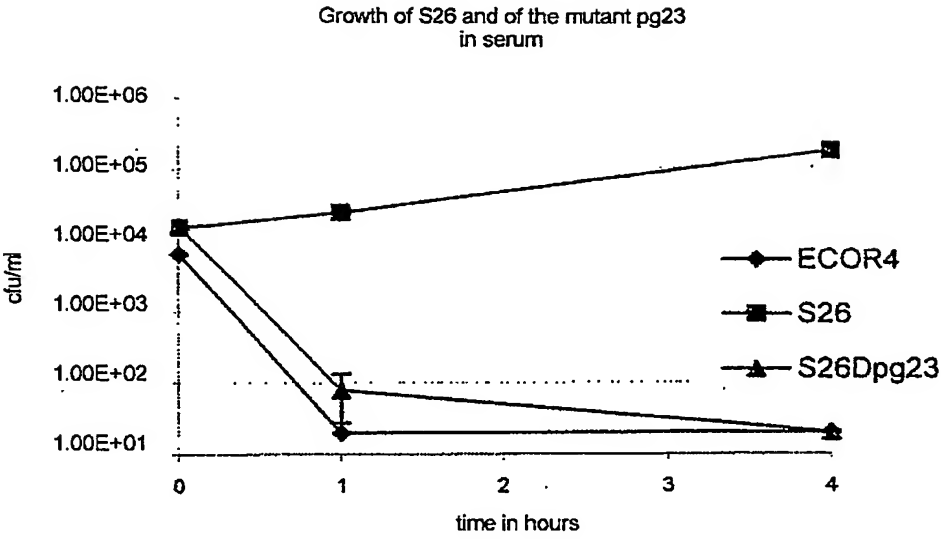
17. Method for inhibiting *in vitro* the proliferation of a pathogenic microorganism in serum, comprising the use of an effective amount of a compound capable of inhibiting the activity, or of reducing the amount, of pathogenicity or virulence target according to any one of claims 6 to 10, or of inhibiting the activity of a peptide according to Claim 15.

18. Method for screening compounds capable of inhibiting the expression of the pathogenicity or virulence target according to any one of claims 6 to 10, or peptides according to claim 15, comprising bringing into contact with the test compound, demonstrating the possible effect of the compound on their activity, and selecting the active compounds.

19. Method for screening compounds capable of inhibiting the biochemical and/or enzyme activity of the peptides expressed by the pathogenicity or virulence target according to any one of claims 6 to 10.

20. Use of the compounds selected according to Claim 19, for developing medicinal products for inhibiting a bacterial infection, in particular an extra-intestinal infection in the case of enterobacteria.

FIGURE 1



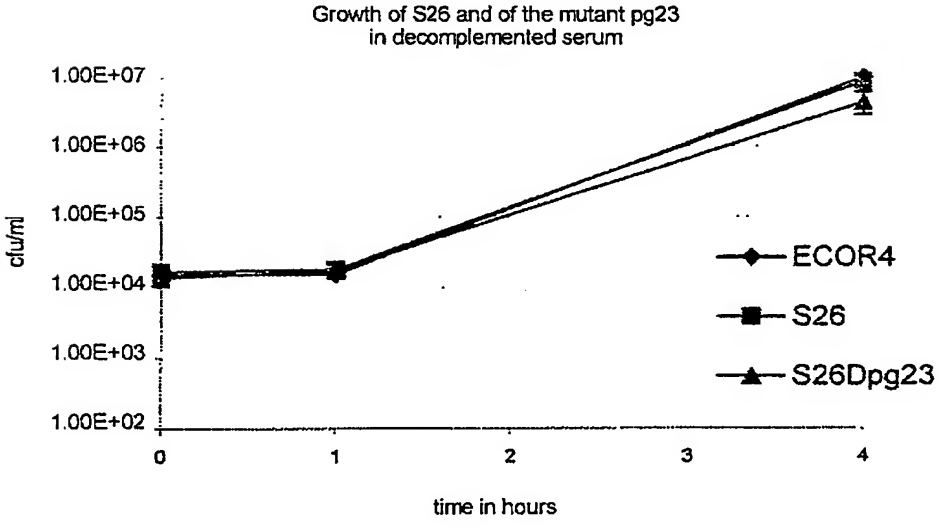


FIGURE 2

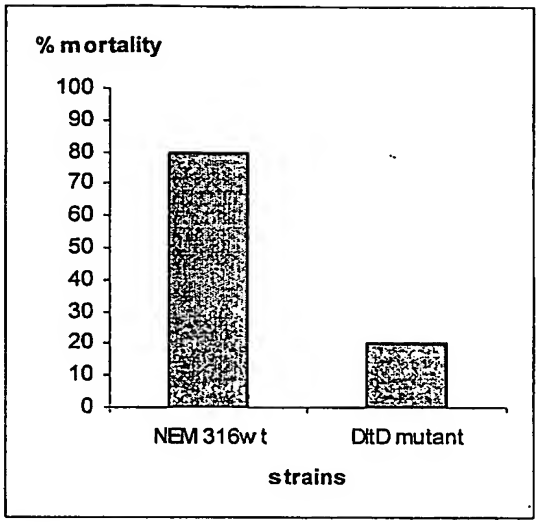

% mortality
100
90
80
70
60
50
40
30
20
10
0
NEM 316 wt
DltD mutant
strains

FIGURE 3

SEQUENCE LISTING

<110> MUTABILIS S.A.
<120> Pathogenicity determinants which can be used as targets for developing means for preventing and controlling bacterial infections and/or systemic dissemination
<130> 1621
<160> 32
<170> PatentIn version 3.1
<210> 1
<211> 305
<212> PRT
<213> Escherichia coli
<400> 1

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1               5                  10                  15

Trp Val Val Glu Glu Gly Phe Ala Gln Ile Pro Ser Trp His Ala Ala
            20                  25                  30

Val Glu Arg Val Ile Pro Val Ala Ile Arg Arg Trp Arg Lys Ala Trp
        35                  40                  45

Phe Ser Ala Pro Ile Lys Ala Glu Arg Lys Ala Phe Arg Glu Ala Leu
    50                  55                  60

Gln Ala Glu Asn Tyr Asp Ala Val Ile Asp Ala Gln Gly Leu Val Lys
65                  70                  75                  80

Ser Ala Ala Leu Val Thr Arg Leu Ala His Gly Val Lys His Gly Leu
                85                  90                  95

Asp Trp Gln Thr Ala Arg Glu Pro Leu Ala Ser Leu Phe Tyr Asn Cys
            100                 105                 110

Lys His His Ile Ala Lys Gln Gln His Ala Val Glu Arg Thr Arg Glu
        115                 120                 125

Leu Phe Ala Lys Ser Leu Gly Tyr Ser Lys Pro Gln Thr Gln Gly Asp
    130                 135                 140

Tyr Ala Ile Ala Gln His Phe Leu Thr Asn Leu Pro Thr Asp Ala Gly
145                 150                 155                 160

Glu Tyr Ala Val Phe Leu His Ala Thr Thr Arg Asp Asp Lys His Trp
                165                 170                 175

Pro Glu Glu His Trp Arg Glu Leu Ile Gly Leu Leu Ala Asp Ser Gly
            180                 185                 190

Ile Arg Ile Lys Leu Pro Trp Gly Ala Pro His Glu Glu Glu Arg Ala
```

```
Lys Arg Leu Ala Glu Gly Phe Ala Tyr Val Glu Val Leu Pro Lys Met
    210                 215                 220

Ser Leu Glu Gly Val Ala Arg Val Leu Ala Gly Ala Lys Phe Val Val
225                 230                 235                 240

Ser Val Asp Thr Gly Leu Ser His Leu Thr Ala Ala Leu Asp Arg Pro
                245                 250                 255

Asn Ile Thr Val Tyr Gly Pro Thr Asp Pro Gly Leu Ile Gly Gly Tyr
            260                 265                 270

Gly Lys Asn Gln Met Val Cys Arg Ala Pro Gly Asn Glu Leu Ser Gln
        275                 280                 285

Leu Thr Ala Asn Ala Val Lys Arg Phe Ile Glu Glu Asn Ala Ala Met
    290                 295                 300

Ile
305
```

<210> 2
<211> 340
<212> PRT
<213> Escherichia coli
<400> 2

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Met Arg Phe His Gly Asp Met Leu Leu Thr Thr Pro Val Ile Ser Ser
1               5                   10                  15

Leu Lys Lys Asn Tyr Pro Asp Ala Lys Ile Asp Val Leu Leu Tyr Gln
            20                  25                  30

Asp Thr Ile Pro Ile Leu Ser Glu Asn Pro Glu Ile Asn Ala Leu Tyr
        35                  40                  45

Gly Ile Lys Asn Lys Lys Ala Lys Ala Ser Glu Lys Ile Ala Asn Phe
    50                  55                  60

Phe His Leu Ile Lys Val Leu Arg Ala Asn Lys Tyr Asp Leu Ile Val
65                  70                  75                  80

Asn Leu Thr Asp Gln Trp Met Val Ala Ile Leu Val Arg Leu Leu Asn
                85                  90                  95

Ala Arg Val Lys Ile Ser Gln Asp Tyr His His Arg Gln Ser Ala Phe
            100                 105                 110

Trp Arg Lys Ser Phe Thr His Leu Val Pro Leu Gln Gly Gly Asn Val
        115                 120                 125
```

```
Val Glu Ser Asn Leu Ser Val Leu Thr Pro Leu Gly Val Asp Ser Leu
    130                 135                 140

Val Lys Gln Thr Thr Met Ser Tyr Pro Pro Ala Ser Trp Lys Arg Met
145                 150                 155                 160

Arg Arg Glu Leu Asp His Ala Gly Val Gly Gln Asn Tyr Val Val Ile
                165                 170                 175

Gln Pro Thr Ala Arg Gln Ile Phe Lys Cys Trp Asp Asn Ala Lys Phe
            180                 185                 190

Ser Ala Val Ile Asp Ala Leu His Ala Arg Gly Tyr Glu Val Val Leu
        195                 200                 205

Thr Ser Gly Pro Asp Lys Asp Asp Leu Ala Cys Val Asn Glu Ile Ala
    210                 215                 220

Gln Gly Cys Gln Thr Pro Pro Val Thr Ala Leu Ala Gly Lys Val Thr
225                 230                 235                 240

Phe Pro Glu Leu Gly Ala Leu Ile Asp His Ala Gln Leu Phe Ile Gly
                245                 250                 255

Val Asp Ser Ala Pro Ala His Ile Ala Ala Ala Val Asn Thr Pro Leu
            260                 265                 270

Ile Ser Leu Phe Gly Ala Thr Asp His Ile Phe Trp Arg Pro Trp Ser
        275                 280                 285

Asn Asn Met Ile Gln Phe Trp Ala Gly Asp Tyr Arg Glu Met Pro Thr
    290                 295                 300

Arg Asp Gln Arg Asp Arg Asn Glu Met Tyr Leu Ser Val Ile Pro Ala
305                 310                 315                 320

Ala Asp Val Ile Ala Ala Val Asp Lys Leu Leu Pro Ser Ser Thr Thr
                325                 330                 335

Gly Thr Ser Leu
            340
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<210> 3
<211> 265
<212> PRT
<213> Escherichia coli
<400> 3

```
Met Val Glu Leu Lys Glu Pro Phe Ala Thr Leu Trp Arg Gly Lys Asp
1               5                   10                  15
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Pro Phe Glu Glu Val Lys Thr Leu Gln Gly Glu Val Phe Arg Glu Leu
20 25 30

Glu Thr Arg Arg Thr Leu Arg Phe Glu Met Ala Gly Lys Ser Tyr Phe
35 40 45

Leu Lys Trp His Arg Gly Thr Thr Leu Lys Glu Ile Ile Lys Asn Leu
50 55 60

Leu Ser Leu Arg Met Pro Val Leu Gly Ala Asp Arg Glu Trp Asn Ala
65 70 75 80

Ile His Arg Leu Arg Asp Val Gly Val Asp Thr Met Tyr Gly Val Ala
85 90 95

Phe Gly Glu Lys Gly Met Asn Pro Leu Thr Arg Thr Ser Phe Ile Ile
100 105 110

Thr Glu Asp Leu Thr Pro Thr Ile Ser Leu Glu Asp Tyr Cys Ala Asp
115 120 125

Trp Ala Thr Asn Pro Pro Asp Val Arg Val Lys Arg Met Leu Ile Lys
130 135 140

Arg Val Ala Thr Met Val Arg Asp Met His Ala Ala Gly Ile Asn His
145 150 155 160

Arg Asp Cys Tyr Ile Cys His Phe Leu Leu His Leu Pro Phe Ser Gly
165 170 175

Lys Glu Glu Glu Leu Lys Ile Ser Val Ile Asp Leu His Arg Ala Gln
180 185 190

Leu Arg Thr Arg Val Pro Arg Arg Trp Arg Asp Lys Asp Leu Ile Gly
195 200 205

Leu Tyr Phe Ser Ser Met Asn Ile Gly Leu Thr Gln Arg Asp Ile Trp
210 215 220

Arg Phe Met Lys Val Tyr Phe Ala Ala Pro Leu Lys Asp Ile Leu Lys
225 230 235 240

Gln Glu Gln Gly Leu Leu Ser Gln Ala Glu Ala Lys Ala Thr Lys Ile
245 250 255

Arg Glu Arg Thr Ile Arg Lys Ser Leu
260 265

<210> 4

<211> 374
<212> PRT
<213> Escherichia coli
<400> 4

Met Ile Val Ala Phe Cys Leu Tyr Lys Tyr Phe Pro Phe Gly Gly Leu
1 5 10 15

Gln Arg Asp Phe Met Arg Ile Ala Gln Thr Val Ala Ala Arg Gly His
20 25 30

His Val Arg Val Tyr Thr Gln Ser Trp Glu Gly Glu Cys Pro Asp Val
35 40 45

Phe Glu Leu Ile Lys Val Pro Val Lys Ser His Thr Asn His Gly Arg
50 55 60

Asn Ala Glu Tyr Phe Ala Trp Val Gln Lys His Leu Arg Glu His Pro
65 70 75 80

Val Asp Lys Val Val Gly Phe Asn Lys Met Pro Gly Leu Asp Val Tyr
85 90 95

Tyr Ala Ala Asp Val Cys Tyr Ala Glu Lys Val Ala Gln Glu Lys Gly
100 105 110

Phe Phe Tyr Arg Leu Thr Ser Arg Tyr Arg His Tyr Ala Ala Phe Glu
115 120 125

Arg Ala Thr Phe Glu Gln Gly Lys Pro Thr Gln Leu Leu Met Leu Thr
130 135 140

Asp Lys Gln Ile Ala Asp Phe Gln Lys His Tyr Gln Thr Glu Ala Glu
145 150 155 160

Arg Phe His Ile Leu Pro Pro Gly Ile Tyr Pro Asp Arg Lys Tyr Ser
165 170 175

Gln Gln Pro Ala Asn Ser Arg Glu Ile Phe Arg Lys Lys Asn Gly Ile
180 185 190

Thr Glu Gln Gln Tyr Leu Leu Leu Gln Val Gly Ser Asp Phe Thr Arg
195 200 205

Lys Gly Val Asp Arg Ser Ile Glu Ala Leu Ala Ser Leu Pro Asp Ser
210 215 220

Leu Arg His Asn Thr Leu Leu Tyr Val Val Gly Gln Asp Lys Pro Arg
225 230 235 240

Lys Phe Glu Ala Leu Ala Glu Lys Arg Gly Val Arg Ser Asn Val His

245 250 255

Phe Phe Ser Gly Arg Asn Asp Val Ser Glu Leu Met Ala Ala Ala Asp
260 265 270

Leu Leu Leu His Pro Ala Tyr Gln Glu Ala Ala Gly Ile Val Leu Leu
275 280 285

Glu Ala Ile Thr Ala Gly Leu Pro Val Leu Thr Thr Ala Val Cys Gly
290 295 300

Tyr Ala His Tyr Ile Val Asp Ala Asn Cys Gly Glu Ala Ile Ala Glu
305 310 315 320

Pro Phe Arg Gln Glu Thr Leu Asn Glu Ile Leu Arg Lys Ala Leu Thr
325 330 335

Gln Ser Ser Leu Arg Gln Ala Trp Ala Glu Asn Ala Arg His Tyr Ala
340 345 350

Asp Thr Gln Asp Leu Tyr Ser Leu Pro Glu Lys Ala Ala Asp Ile Ile
355 360 365

Thr Gly Gly Leu Asp Gly
370

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<211> 348
<212> PRT
<213> Escherichia coli
<400> 5

Met Lys Ile Leu Val Ile Gly Pro Ser Trp Val Gly Asp Met Met Met
1 5 10 15

Ser Gln Ser Leu Tyr Arg Thr Leu Gln Ala Arg Tyr Pro Gln Ala Ile
20 25 30

Ile Asp Val Met Ala Pro Ala Trp Cys Arg Pro Leu Leu Ser Arg Met
35 40 45

Pro Glu Val Asn Glu Ala Ile Pro Met Pro Leu Gly His Gly Ala Leu
50 55 60

Glu Ile Gly Glu Arg Arg Lys Leu Gly His Ser Leu Arg Glu Lys Arg
65 70 75 80

Tyr Asp Arg Ala Tyr Val Leu Pro Asn Ser Phe Lys Ser Ala Leu Val
85 90 95

Pro Phe Phe Ala Gly Ile Pro His Arg Thr Gly Trp Arg Gly Glu Met

100 105 110

Arg Tyr Gly Leu Leu Asn Asp Val Arg Val Leu Asp Lys Glu Ala Trp
115 120 125

Pro Leu Met Val Glu Arg Tyr Ile Ala Leu Ala Tyr Asp Lys Gly Ile
130 135 140

Met Arg Thr Ala Gln Asp Leu Pro Gln Pro Leu Leu Trp Pro Gln Leu
145 150 155 160

Gln Val Ser Glu Gly Glu Lys Ser Tyr Thr Cys Asn Gln Phe Ser Leu
165 170 175

Ser Ser Glu Arg Pro Met Ile Gly Phe Cys Pro Gly Ala Glu Phe Gly
180 185 190

Pro Ala Lys Arg Trp Pro His Tyr His Tyr Ala Glu Leu Ala Lys Gln
195 200 205

Leu Ile Asp Glu Gly Tyr Gln Val Val Leu Phe Gly Ser Ala Lys Asp
210 215 220

His Glu Ala Gly Asn Glu Ile Leu Ala Ala Leu Asn Thr Glu Gln Gln
225 230 235 240

Ala Trp Cys Arg Asn Leu Ala Gly Glu Thr Gln Leu Asp Gln Ala Val
245 250 255

Ile Leu Ile Ala Ala Cys Lys Ala Ile Val Thr Asn Asp Ser Gly Leu
260 265 270

Met His Val Ala Ala Ala Leu Asn Arg Pro Leu Val Ala Leu Tyr Gly
275 280 285

Pro Ser Ser Pro Asp Phe Thr Pro Pro Leu Ser His Lys Ala Arg Val
290 295 300

Ile Arg Leu Ile Thr Gly Tyr His Lys Val Arg Lys Gly Asp Ala Ala
305 310 315 320

Glu Gly Tyr His Gln Ser Leu Ile Asp Ile Thr Pro Gln Arg Val Leu
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Glu Glu Leu Asn Ala Leu Leu Leu Gln Glu Glu Ala
340 345

<210> 6
<211> 338
<212> PRT

<213> Escherichia coli
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Met Ser Ala His Tyr Phe Asn Pro Gln Glu Met Ile Asn Lys Thr Ile
1 5 10 15

Ile Phe Asp Glu Arg Pro Ala Ala Ser Val Ala Ser Ser Phe His Val
20 25 30

Ala Tyr Gly Ile Asp Lys Asn Phe Leu Phe Gly Cys Gly Val Ser Ile
35 40 45

Thr Ser Val Leu Leu His Asn Asn Asp Val Ser Phe Val Phe His Val
50 55 60

Phe Ile Asp Asp Ile Pro Glu Ala Asp Ile Gln Arg Leu Ala Gln Leu
65 70 75 80

Ala Lys Ser Tyr Arg Thr Cys Ile Gln Ile His Leu Val Asn Cys Glu
85 90 95

Arg Leu Lys Ala Leu Pro Thr Thr Lys Asn Trp Ser Ile Ala Met Tyr
100 105 110

Phe Arg Phe Val Ile Ala Asp Tyr Phe Ile Asp Gln Gln Asp Lys Ile
115 120 125

Leu Tyr Leu Asp Ala Asp Ile Ala Cys Gln Gly Asn Leu Lys Pro Leu
130 135 140

Ile Thr Met Asp Leu Ala Asn Asn Val Ala Ala Val Val Thr Glu Arg
145 150 155 160

Asp Ala Asn Trp Trp Ser Leu Arg Gly Gln Ser Leu Gln Cys Asn Glu
165 170 175

Leu Glu Lys Gly Tyr Phe Asn Ser Gly Val Leu Leu Ile Asn Thr Leu
180 185 190

Ala Trp Ala Gln Glu Ser Val Ser Ala Lys Ala Met Ser Met Leu Ala
195 200 205

Asp Lys Ala Ile Val Ser Arg Leu Thr Tyr Met Asp Gln Asp Ile Leu
210 215 220

Asn Leu Ile Leu Leu Gly Lys Val Lys Phe Ile Asp Ala Lys Tyr Asn
225 230 235 240

Thr Gln Phe Ser Leu Asn Tyr Glu Leu Lys Lys Ser Phe Val Cys Pro
245 250 255

```
Ile Asn Asp Glu Thr Val Leu Ile His Tyr Val Gly Pro Thr Lys Pro
            260                 265                 270

Trp His Tyr Trp Ala Gly Tyr Pro Ser Ala Gln Pro Phe Ile Lys Ala
        275                 280                 285

Lys Glu Ala Ser Pro Trp Lys Asn Glu Pro Leu Met Arg Pro Val Asn
    290                 295                 300

Ser Asn Tyr Ala Arg Tyr Cys Ala Lys His Asn Phe Lys Gln Asn Lys
305                 310                 315                 320

Pro Ile Asn Gly Ile Met Asn Tyr Ile Tyr Tyr Phe Tyr Leu Lys Ile
            325                 330                 335

Ile Lys
```

<210> 7
<211> 302
<212> PRT
<213> Escherichia coli
<400> 7

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Met Ala Ala Ile Asn Thr Lys Val Lys Lys Ala Val Ile Pro Val Ala
1               5                   10                  15

Gly Leu Gly Thr Arg Met Leu Pro Ala Thr Lys Ala Ile Pro Lys Glu
            20                  25                  30

Met Leu Pro Leu Val Asp Lys Pro Leu Ile Gln Tyr Val Val Asn Glu
        35                  40                  45

Cys Ile Ala Ala Gly Ile Thr Glu Ile Val Leu Val Thr His Ser Ser
    50                  55                  60

Lys Asn Ser Ile Glu Asn His Phe Asp Thr Ser Phe Glu Leu Glu Ala
65                  70                  75                  80

Met Leu Glu Lys Arg Val Lys Arg Gln Leu Leu Asp Glu Val Gln Ser
                85                  90                  95

Ile Cys Pro Pro His Val Thr Ile Met Gln Val Arg Gln Gly Leu Ala
            100                 105                 110

Lys Gly Leu Gly His Ala Val Leu Cys Ala His Pro Val Val Gly Asp
        115                 120                 125

Glu Pro Val Ala Val Ile Leu Pro Asp Val Ile Leu Asp Glu Tyr Glu
    130                 135                 140
```

Ser Asp Leu Ser Gln Asp Asn Leu Ala Glu Met Ile Arg Arg Phe Asp
145 150 155 160

Glu Thr Gly His Ser Gln Ile Met Val Glu Pro Val Ala Asp Val Thr
165 170 175

Ala Tyr Gly Val Val Asp Cys Lys Gly Val Glu Leu Ala Pro Gly Glu
180 185 190

Ser Val Pro Met Val Gly Val Val Glu Lys Pro Lys Ala Asp Val Ala
195 200 205

Pro Ser Asn Leu Ala Ile Val Gly Arg Tyr Val Leu Ser Ala Asp Ile
210 215 220

Trp Pro Leu Leu Ala Lys Thr Pro Pro Gly Ala Gly Asp Glu Ile Gln
225 230 235 240

Leu Thr Asp Ala Ile Asp Met Leu Ile Glu Lys Glu Thr Val Glu Ala
245 250 255

Tyr His Met Lys Gly Lys Ser His Asp Cys Gly Asn Lys Leu Gly Tyr
260 265 270

Met Gln Ala Phe Val Glu Tyr Gly Ile Arg His Asn Thr Leu Gly Thr
275 280 285

Glu Phe Lys Ala Trp Leu Glu Glu Glu Met Gly Ile Lys Lys
290 295 300

<210> 8
<211> 546
<212> PRT
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<400> 8

Met Ala Ile His Asn Arg Ala Gly Gln Pro Ala Gln Gln Ser Asp Leu
1 5 10 15

Ile Asn Val Ala Gln Leu Thr Ala Gln Tyr Tyr Val Leu Lys Pro Glu
20 25 30

Ala Gly Asn Ala Glu His Ala Val Lys Phe Gly Thr Ser Gly His Arg
35 40 45

Gly Ser Ala Ala Arg His Ser Phe Asn Glu Pro His Ile Leu Ala Ile
50 55 60

Ala Gln Ala Ile Ala Glu Glu Arg Ala Lys Asn Gly Ile Thr Gly Pro
65 70 75 80

Cys Tyr Val Gly Lys Asp Thr His Ala Leu Ser Glu Pro Ala Phe Ile
85 90 95

Ser Val Leu Glu Val Leu Ala Ala Asn Gly Val Asp Val Ile Val Gln
100 105 110

Glu Asn Asn Gly Phe Thr Pro Thr Pro Ala Val Ser Asn Ala Ile Leu
115 120 125

Val His Asn Lys Lys Gly Gly Pro Leu Ala Asp Gly Ile Val Ile Thr
130 135 140

Pro Ser His Asn Pro Pro Glu Asp Gly Gly Ile Lys Tyr Asn Pro Pro
145 150 155 160

Asn Gly Gly Pro Ala Asp Thr Asn Val Thr Lys Val Val Glu Asp Arg
165 170 175

Ala Asn Ala Leu Leu Ala Asp Gly Leu Lys Gly Val Lys Arg Ile Ser
180 185 190

Leu Asp Glu Ala Met Ala Ser Gly His Val Lys Glu Gln Asp Leu Val
195 200 205

Gln Pro Phe Val Glu Gly Leu Ala Asp Ile Val Asp Met Ala Ala Ile
210 215 220

Gln Lys Ala Gly Leu Thr Leu Gly Val Asp Pro Leu Gly Gly Ser Gly
225 230 235 240

Ile Glu Tyr Trp Lys Arg Ile Gly Glu Tyr Tyr Asn Leu Asn Leu Thr
245 250 255

Ile Val Asn Asp Gln Val Asp Gln Thr Phe Arg Phe Met His Leu Asp
260 265 270

Lys Asp Gly Ala Ile Arg Met Asp Cys Ser Ser Glu Cys Ala Met Ala
275 280 285

Gly Leu Leu Ala Leu Arg Asp Lys Phe Asp Leu Ala Phe Ala Asn Asp
290 295 300

Pro Asp Tyr Asp Arg His Gly Ile Val Thr Pro Ala Gly Leu Met Asn
305 310 315 320

Pro Asn His Tyr Leu Ala Val Ala Ile Asn Tyr Leu Phe Gln His Arg
325 330 335

Pro Gln Trp Gly Lys Asp Val Ala Val Gly Lys Thr Leu Val Ser Ser

340 345 350

Ala Met Ile Asp Arg Val Val Asn Asp Leu Gly Arg Lys Leu Val Glu
355 360 365

Val Pro Val Gly Phe Lys Trp Phe Val Asp Gly Leu Phe Asp Gly Ser
370 375 380

Phe Gly Phe Gly Gly Glu Glu Ser Ala Gly Ala Ser Phe Leu Arg Phe
385 390 395 400

Asp Gly Thr Pro Trp Ser Thr Asp Lys Asp Gly Ile Ile Met Cys Leu
405 410 415

Leu Ala Ala Glu Ile Thr Ala Val Thr Gly Lys Asn Pro Gln Glu His
420 425 430

Tyr Asn Glu Leu Ala Lys Arg Phe Gly Ala Pro Ser Tyr Asn Arg Leu
435 440 445

Gln Ala Ala Ala Thr Ser Ala Gln Lys Ala Ala Leu Ser Lys Leu Ser
450 455 460

Pro Glu Met Val Ser Ala Ser Thr Leu Ala Gly Asp Pro Ile Thr Ala
465 470 475 480

Arg Leu Thr Ala Ala Pro Gly Asn Gly Ala Ser Ile Gly Gly Leu Lys
485 490 495

Val Met Thr Asp Asn Gly Trp Phe Ala Ala Arg Pro Ser Gly Thr Glu
500 505 510

Asp Ala Tyr Lys Ile Tyr Cys Glu Ser Phe Leu Gly Glu Glu His Arg
515 520 525

Lys Gln Ile Glu Lys Glu Ala Val Glu Ile Val Ser Glu Val Leu Lys
530 535 540

Asn Ala
545

<210> 9
<211> 558
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<213> Escherichia coli
<400> 9

Met Lys Leu Phe Lys Ser Ile Leu Leu Ile Ala Ala Cys His Ala Ala
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Gln Ala Ser Ala Ala Ile Asp Ile Asn Ala Asp Pro Asn Leu Thr Gly

20 25 30

Ala Ala Pro Leu Thr Gly Ile Leu Asn Gly Gln Gln Ser Asp Thr Gln
35 40 45

Asn Met Ser Gly Phe Asp Asn Thr Pro Pro Pro Ser Pro Pro Val Val
50 55 60

Met Ser Arg Met Phe Gly Ala Gln Leu Phe Asn Gly Thr Ser Ala Asp
65 70 75 80

Ser Gly Ala Thr Val Gly Phe Asn Pro Asp Tyr Ile Leu Asn Pro Gly
85 90 95

Asp Ser Ile Gln Val Arg Leu Trp Gly Ala Phe Thr Phe Asp Gly Ala
100 105 110

Leu Gln Val Asp Pro Lys Gly Asn Ile Phe Leu Pro Asn Val Gly Pro
115 120 125

Val Lys Val Ala Gly Val Ser Asn Ser Gln Leu Asn Ala Leu Val Thr
130 135 140

Ser Lys Val Lys Glu Val Tyr Gln Ser Asn Val Asn Val Tyr Ala Ser
145 150 155 160

Leu Leu Gln Ala Gln Pro Val Lys Val Tyr Val Thr Gly Phe Val Arg
165 170 175

Asn Pro Gly Leu Tyr Gly Gly Val Thr Ser Asp Ser Leu Leu Asn Tyr
180 185 190

Leu Ile Lys Ala Gly Gly Val Asp Pro Glu Arg Gly Ser Tyr Val Asp
195 200 205

Ile Val Val Lys Arg Gly Asn Arg Val Arg Ser Asn Val Asn Leu Tyr
210 215 220

Asp Phe Leu Leu Asn Gly Lys Leu Gly Leu Ser Gln Phe Ala Asp Gly
225 230 235 240

Asp Thr Ile Ile Val Gly Pro Arg Gln His Thr Phe Ser Val Gln Gly
245 250 255

Asp Val Phe Asn Ser Tyr Asp Phe Glu Phe Arg Glu Ser Ser Ile Pro
260 265 270

Val Thr Glu Ala Leu Ser Trp Ala Arg Pro Lys Pro Gly Ala Thr His
275 280 285

Ile Thr Ile Met Arg Lys Gln Gly Leu Gln Lys Arg Ser Glu Tyr Tyr
290 295 300

Pro Ile Ser Ser Ala Pro Gly Arg Met Leu Gln Asn Gly Asp Thr Leu
305 310 315 320

Ile Val Ser Thr Asp Arg Tyr Ala Gly Thr Ile Gln Val Arg Val Glu
325 330 335

Gly Ala His Ser Gly Glu His Ala Met Val Leu Pro Tyr Gly Ser Thr
340 345 350

Met Arg Ala Val Leu Glu Lys Val Arg Pro Asn Ser Met Ser Gln Met
355 360 365

Asn Ala Val Gln Leu Tyr Arg Pro Ser Val Ala Gln Arg Gln Lys Glu
370 375 380

Met Leu Asn Leu Ser Leu Gln Lys Leu Glu Glu Ala Ser Leu Ser Ala
385 390 395 400

Gln Ser Ser Thr Lys Glu Glu Ala Ser Leu Arg Met Gln Glu Ala Gln
405 410 415

Leu Ile Ser Arg Phe Val Ala Lys Ala Arg Thr Val Val Pro Lys Gly
420 425 430

Glu Val Ile Leu Asn Glu Ser Asn Ile Asp Ser Val Leu Leu Glu Asp
435 440 445

Gly Asp Val Ile Asn Ile Pro Glu Lys Thr Ser Leu Val Met Val His
450 455 460

Gly Glu Val Leu Phe Pro Asn Ala Val Ser Trp Gln Lys Gly Met Thr
465 470 475 480

Thr Glu Asp Tyr Ile Glu Lys Cys Gly Gly Leu Thr Gln Lys Ser Gly
485 490 495

Asn Ala Arg Ile Ile Val Ile Arg Gln Asn Gly Ala Ala Val Asn Ala
500 505 510

Glu Asp Val Asp Ser Leu Lys Pro Gly Asp Glu Ile Met Val Leu Pro
515 520 525

Lys Tyr Glu Ser Lys Asn Ile Glu Val Thr Arg Gly Ile Ser Thr Ile
530 535 540

Leu Tyr Gln Leu Ala Val Gly Ala Lys Val Ile Leu Ser Leu

545 550 555

<210> 10
<211> 207
<212> PRT
<213> Escherichia coli
<400> 10

Met Ser Lys Lys Leu Ile Ile Phe Gly Ala Gly Gly Phe Ser Lys Ser
1 5 10 15

Ile Ile Asp Ser Leu Asn His Lys His Tyr Glu Leu Ile Gly Phe Ile
20 25 30

Asp Lys Tyr Lys Ser Gly Tyr His Gln Ser Tyr Pro Ile Leu Gly Asn
35 40 45

Asp Ile Ala Asp Ile Glu Asn Lys Asp Asn Tyr Tyr Tyr Phe Ile Gly
50 55 60

Ile Gly Lys Pro Ser Thr Arg Lys His Tyr Leu Asn Ile Ile Arg Lys
65 70 75 80

His Asn Leu Arg Leu Ile Asn Ile Ile Asp Lys Thr Ala Ile Leu Ser
85 90 95

Pro Asn Ile Ile Leu Gly Asp Gly Ile Phe Ile Gly Lys Met Cys Ile
100 105 110

Leu Asn Arg Asp Thr Arg Ile His Asp Ala Val Val Ile Asn Thr Arg
115 120 125

Ser Leu Ile Glu His Gly Asn Glu Ile Gly Cys Cys Ser Asn Ile Ser
130 135 140

Thr Asn Val Val Leu Asn Gly Asp Val Ser Val Gly Glu Glu Thr Phe
145 150 155 160

Val Gly Ser Val Thr Val Val Asn Gly Gln Leu Lys Leu Gly Ser Lys
165 170 175

Ser Ile Ile Gly Ser Gly Ser Val Val Ile Arg Asn Ile Pro Ser Asn
180 185 190

Val Val Val Ala Gly Thr Pro Thr Arg Leu Ile Arg Gly Asn Glu
195 200 205

<210> 11
<211> 191
<212> PRT
<213> Escherichia coli
<400> 11

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Met Ala Lys Ser Val Pro Ala Ile Phe Leu Asp Arg Asp Gly Thr Ile
1               5                  10                  15

Asn Val Asp His Gly Tyr Val His Glu Ile Asp Asn Phe Glu Phe Ile
            20                  25                  30

Asp Gly Val Ile Asp Ala Met Arg Glu Leu Lys Lys Met Gly Phe Ala
        35                  40                  45

Leu Val Val Val Thr Asn Gln Ser Gly Ile Ala Arg Gly Lys Phe Thr
    50                  55                  60

Glu Ala Gln Phe Glu Thr Leu Thr Glu Trp Met Asp Trp Ser Leu Ala
65                  70                  75                  80

Asp Arg Asp Val Asp Leu Asp Gly Ile Tyr Tyr Cys Pro His His Pro
                85                  90                  95

Gln Gly Ser Val Glu Glu Phe Arg Gln Val Cys Asp Cys Arg Lys Pro
            100                 105                 110

His Pro Gly Met Leu Leu Ser Ala Arg Asp Tyr Leu His Ile Asp Met
        115                 120                 125

Ala Ala Ser Tyr Met Val Gly Asp Lys Leu Glu Asp Met Gln Ala Ala
    130                 135                 140

Val Ala Ala Asn Val Gly Thr Lys Val Leu Val Arg Thr Gly Lys Pro
145                 150                 155                 160

Ile Thr Pro Glu Ala Glu Asn Ala Ala Asp Trp Val Leu Asn Ser Leu
                165                 170                 175

Ala Asp Leu Pro Gln Ala Ile Lys Lys Gln Gln Lys Pro Ala Gln
            180                 185                 190
```

<210> 12
<211> 310
<212> PRT
<213> Escherichia coli
<400> 12

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Met Ile Ile Val Thr Gly Gly Ala Gly Phe Ile Gly Ser Asn Ile Val
1               5                  10                  15

Lys Ala Leu Asn Asp Lys Gly Ile Thr Asp Ile Leu Val Val Asp Asn
            20                  25                  30

Leu Lys Asp Gly Thr Lys Phe Val Asn Leu Val Asp Leu Asn Ile Ala
        35                  40                  45
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Asp Tyr Met Asp Lys Glu Asp Phe Leu Ile Gln Ile Met Ala Gly Glu
50 55 60

Glu Phe Gly Asp Val Glu Ala Ile Phe His Glu Gly Ala Cys Ser Ser
65 70 75 80

Thr Thr Glu Trp Asp Gly Lys Tyr Met Met Asp Asn Asn Tyr Gln Tyr
85 90 95

Ser Lys Glu Leu Leu His Tyr Cys Leu Glu Arg Glu Ile Pro Phe Leu
100 105 110

Tyr Ala Ser Ser Ala Ala Thr Tyr Gly Gly Arg Thr Ser Asp Phe Ile
115 120 125

Glu Ser Arg Glu Tyr Glu Lys Pro Leu Asn Val Tyr Gly Tyr Ser Lys
130 135 140

Phe Leu Phe Asp Glu Tyr Val Arg Gln Ile Leu Pro Glu Ala Asn Ser
145 150 155 160

Gln Ile Val Gly Phe Arg Tyr Phe Asn Val Tyr Gly Pro Arg Glu Gly
165 170 175

His Lys Gly Ser Met Ala Ser Val Ala Phe His Leu Asn Thr Gln Leu
180 185 190

Asn Asn Gly Glu Ser Pro Lys Leu Phe Glu Gly Ser Glu Asn Phe Lys
195 200 205

Arg Asp Phe Val Tyr Val Gly Asp Val Ala Asp Val Asn Leu Trp Phe
210 215 220

Leu Glu Asn Gly Val Ser Gly Ile Phe Asn Leu Gly Thr Gly Arg Ala
225 230 235 240

Glu Ser Phe Gln Ala Val Ala Asp Ala Thr Leu Ala Tyr His Lys Lys
245 250 255

Gly Gln Ile Glu Tyr Ile Pro Phe Pro Asp Lys Leu Lys Gly Arg Tyr
260 265 270

Gln Ala Phe Thr Gln Ala Asp Leu Thr Asn Leu Arg Ala Ala Gly Tyr
275 280 285

Asp Lys Pro Phe Lys Thr Val Ala Glu Gly Val Thr Glu Tyr Met Ala
290 295 300

Trp Leu Asn Arg Asp Ala

305 310

<210> 13
<211> 477
<212> PRT
<213> Escherichia coli
<400> 13

Met Lys Val Thr Leu Pro Glu Phe Glu Arg Ala Gly Val Met Val Val
1 5 10 15

Gly Asp Val Met Leu Asp Arg Tyr Trp Tyr Gly Pro Thr Ser Arg Ile
20 25 30

Ser Pro Glu Ala Pro Val Pro Val Val Lys Val Asn Thr Ile Glu Glu
35 40 45

Arg Pro Gly Gly Ala Ala Asn Val Ala Met Asn Ile Ala Ser Leu Gly
50 55 60

Ala Asn Ala Arg Leu Val Gly Leu Thr Gly Ile Asp Asp Ala Ala Arg
65 70 75 80

Ala Leu Ser Lys Ser Leu Ala Asp Val Asn Val Lys Cys Asp Phe Val
85 90 95

Ser Val Pro Thr His Pro Thr Ile Thr Lys Leu Arg Val Leu Ser Arg
100 105 110

Asn Gln Gln Leu Ile Arg Leu Asp Phe Glu Glu Gly Phe Glu Gly Val
115 120 125

Asp Pro Gln Pro Leu His Glu Arg Ile Asn Gln Ala Leu Ser Ser Ile
130 135 140

Gly Ala Leu Val Leu Ser Asp Tyr Ala Lys Gly Ala Leu Ala Ser Val
145 150 155 160

Gln Gln Met Ile Gln Leu Ala Arg Lys Ala Gly Val Pro Val Leu Ile
165 170 175

Asp Pro Lys Gly Thr Asp Phe Glu Arg Tyr Arg Gly Ala Thr Leu Leu
180 185 190

Thr Pro Asn Leu Ser Glu Phe Glu Ala Val Val Gly Lys Cys Lys Thr
195 200 205

Glu Glu Glu Ile Val Glu Arg Gly Met Lys Leu Ile Ala Asp Tyr Glu
210 215 220

Leu Ser Ala Leu Leu Val Thr Arg Ser Glu Gln Gly Met Ser Leu Leu

225 230 235 240

Gln Pro Gly Lys Ala Pro Leu His Met Pro Thr Gln Ala Gln Glu Val
245 250 255

Tyr Asp Val Thr Gly Ala Gly Asp Thr Val Ile Gly Val Leu Ala Ala
260 265 270

Thr Leu Ala Ala Gly Asn Ser Leu Glu Glu Ala Cys Phe Phe Ala Asn
275 280 285

Ala Ala Ala Gly Val Val Val Gly Lys Leu Gly Thr Ser Thr Val Ser
290 295 300

Pro Ile Glu Leu Glu Asn Ala Val Arg Gly Arg Ala Asp Thr Gly Phe
305 310 315 320

Gly Val Met Thr Glu Glu Glu Leu Lys Leu Ala Val Ala Ala Ala Arg
325 330 335

Lys Arg Gly Glu Lys Val Val Met Thr Asn Gly Val Phe Asp Ile Leu
340 345 350

His Ala Gly His Val Ser Tyr Leu Ala Asn Ala Arg Lys Leu Gly Asp
355 360 365

Arg Leu Ile Val Ala Val Asn Ser Asp Ala Ser Thr Lys Arg Leu Lys
370 375 380

Gly Asp Ser Arg Pro Val Asn Pro Leu Glu Gln Arg Met Ile Val Leu
385 390 395 400

Gly Ala Leu Glu Ala Val Asp Trp Val Val Ser Phe Glu Glu Asp Thr
405 410 415

Pro Gln Arg Leu Ile Ala Gly Ile Leu Pro Asp Leu Leu Val Lys Gly
420 425 430

Gly Asp Tyr Lys Pro Glu Glu Ile Ala Gly Ser Lys Glu Val Trp Ala
435 440 445

Asn Gly Gly Glu Val Leu Val Leu Asn Phe Glu Asp Gly Cys Ser Thr
450 455 460

Thr Asn Ile Ile Lys Lys Ile Gln Gln Asp Lys Lys Gly
465 470 475

<210> 14
<211> 420
<212> PRT

<213> Escherichia coli
<400> 14

Met Leu Lys Arg Leu Gly Lys Val Phe Gly Pro Leu Val Cys Ala Leu
1 5 10 15

Leu Leu Leu Val Gly Leu Tyr Leu Val Phe Pro Val Ser Gln Pro His
20 25 30

His Leu Gly Lys Glu Lys Asn Ser Ala Val Ala Leu Thr Lys Ala Gly
35 40 45

Phe Lys Ser Arg Val Gln Lys Val Arg Ala Phe Ser Asp Pro Lys Ala
50 55 60

Asn Phe Val Pro Phe Phe Gly Ser Ser Glu Trp Leu Arg Phe Asp Ala
65 70 75 80

Met His Pro Ser Val Leu Ala Glu Ala Tyr Lys Arg Pro Tyr Ile Pro
85 90 95

Tyr Leu Leu Gly Gln Lys Gly Ala Ala Ser Leu Thr Gln Tyr Tyr Gly
100 105 110

Ile Gln Gln Ile Lys Gly Gln Ile Lys Asn Lys Lys Ala Ile Tyr Val
115 120 125

Ile Ser Pro Gln Trp Phe Val Arg Lys Gly Ala Asn Lys Gly Ala Phe
130 135 140

Gln Asn Tyr Phe Ser Asn Asp Gln Thr Ile Arg Phe Leu Gln Asn Gln
145 150 155 160

Thr Gly Thr Thr Tyr Asp Arg Tyr Ala Ala Arg Arg Leu Leu Lys Leu
165 170 175

Tyr Pro Glu Ala Ser Met Ser Asp Leu Ile Glu Lys Val Ala Asp Gly
180 185 190

Gln Lys Leu Ser Asn Lys Asp Lys Gln Arg Leu Lys Phe Asn Asp Trp
195 200 205

Val Phe Glu Lys Thr Asp Ala Ile Phe Ser Tyr Leu Pro Leu Gly Lys
210 215 220

Thr Tyr Asn Gln Val Ile Met Pro His Val Gly Lys Leu Pro Lys Ala
225 230 235 240

Phe Ser Tyr Asn His Leu Ser Arg Ile Ala Ser Gln Asp Ala Lys Val
245 250 255

Ala Thr Arg Ser Asn Gln Phe Gly Ile Asp Asp Arg Phe Tyr Gln Thr
260 265 270

Arg Ile Lys Lys His Leu Lys Lys Leu Lys Gly Ser Gln Arg His Phe
275 280 285

Asn Tyr Thr Lys Ser Pro Glu Phe Asn Asp Leu Gln Leu Val Leu Asn
290 295 300

Glu Phe Ser Lys Gln Asn Thr Asp Val Leu Phe Val Ile Pro Pro Val
305 310 315 320

Asn Lys Lys Trp Thr Asp Tyr Thr Gly Leu Asp Gln Lys Met Tyr Gln
325 330 335

Lys Ser Val Glu Lys Ile Lys His Gln Leu Gln Ser Gln Gly Phe Asn
340 345 350

His Ile Ser Asp Leu Ser Arg Asp Gly Gly Lys Pro Tyr Phe Met Gln
355 360 365

Asp Thr Ile His Leu Gly Trp Asn Gly Trp Leu Glu Leu Asp Lys His
370 375 380

Ile Asn Pro Phe Leu Thr Glu Glu Asn Ser Lys Pro Asn Tyr His Ile
385 390 395 400

Asn Asn Lys Phe Leu Lys Arg Ser Trp Ala Lys Tyr Thr Gly Arg Pro
405 410 415

Ser Asp Tyr Lys
420

<210> 15
<211> 511
<212> PRT
<213> Escherichia coli
<400> 15

Met Ile His Asp Met Ile Lys Thr Ile Glu His Phe Ala Glu Thr Gln
1 5 10 15

Ala Asp Phe Pro Val Tyr Asp Ile Leu Gly Glu Val His Thr Tyr Gly
20 25 30

Gln Leu Lys Val Asp Ser Asp Ser Leu Ala Ala His Ile Asp Ser Leu
35 40 45

Gly Leu Val Glu Lys Ser Pro Val Leu Val Phe Gly Gly Gln Glu Tyr
50 55 60

```
Glu Met Leu Ala Thr Phe Val Ala Leu Thr Lys Ser Gly His Ala Tyr
65                  70                  75                  80

Ile Pro Val Asp Gln His Ser Ala Leu Asp Arg Ile Gln Ala Ile Met
                85                  90                  95

Thr Val Ala Gln Pro Ser Leu Ile Ile Ser Ile Gly Glu Phe Pro Leu
            100                 105                 110

Glu Val Asp Asn Val Pro Ile Leu Asp Val Ser Gln Val Ser Ala Ile
        115                 120                 125

Phe Glu Glu Lys Thr Pro Tyr Glu Val Thr His Ser Val Lys Gly Asp
    130                 135                 140

Asp Asn Tyr Tyr Ile Ile Phe Thr Ser Gly Thr Thr Gly Leu Pro Lys
145                 150                 155                 160

Gly Val Gln Ile Ser His Asp Asn Leu Leu Ser Phe Thr Asn Trp Met
                165                 170                 175

Ile Ser Asp Asp Glu Phe Ser Val Pro Glu Arg Pro Gln Met Leu Ala
            180                 185                 190

Gln Pro Pro Tyr Ser Phe Asp Leu Ser Val Met Tyr Trp Ala Pro Thr
        195                 200                 205

Leu Ala Met Gly Gly Thr Leu Phe Ala Leu Pro Lys Thr Val Val Asn
    210                 215                 220

Asp Phe Lys Lys Leu Phe Ala Thr Ile Asn Glu Leu Pro Ile Gln Val
225                 230                 235                 240

Trp Thr Ser Thr Pro Ser Phe Ala Asp Met Ala Leu Leu Ser Asn Asp
                245                 250                 255

Phe Asn Ser Glu Thr Leu Pro Gln Leu Thr His Phe Tyr Phe Asp Gly
            260                 265                 270

Glu Glu Leu Thr Val Lys Thr Ala Gln Lys Leu Arg Gln Arg Phe Pro
        275                 280                 285

Lys Ala Arg Ile Val Asn Ala Tyr Gly Pro Thr Glu Ala Thr Val Ala
    290                 295                 300

Leu Ser Ala Val Ala Ile Thr Asp Glu Met Leu Glu Thr Cys Lys Arg
305                 310                 315                 320

Leu Pro Ile Gly Tyr Thr Lys Asp Asp Ser Pro Thr Tyr Val Ile Asp
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325 330 335

Glu Glu Gly His Lys Leu Pro Asn Gly Glu Gln Gly Glu Ile Ile Ile
340 345 350

Ala Gly Pro Ala Val Ser Lys Gly Tyr Leu Asn Asn Pro Glu Lys Thr
355 360 365

Ala Glu Ala Phe Phe Gln Phe Glu Gly Leu Pro Ala Tyr His Thr Gly
370 375 380

Asp Leu Gly Ser Met Thr Asp Glu Gly Leu Leu Leu Tyr Gly Gly Arg
385 390 395 400

Met Asp Phe Gln Ile Lys Phe Asn Gly Tyr Arg Ile Glu Leu Glu Asp
405 410 415

Val Ser Gln Asn Leu Asn Lys Ser Gln Tyr Val Lys Ser Ala Val Ala
420 425 430

Val Pro Arg Tyr Asn Lys Asp His Lys Val Gln Asn Leu Leu Ala Tyr
435 440 445

Ile Val Leu Lys Glu Gly Val Arg Asp Asp Phe Glu Arg Asp Leu Asp
450 455 460

Leu Thr Lys Ala Ile Lys Glu Asp Leu Lys Asp Ile Met Met Asp Tyr
465 470 475 480

Met Met Pro Ser Lys Phe Ile Tyr Arg Glu Asp Leu Pro Leu Thr Pro
485 490 495

Asn Gly Lys Ile Asp Ile Lys Gly Leu Met Ser Glu Val Asn Lys
500 505 510

<210> 16
<211> 919
<212> DNA
<213> Escherichia coli
<400> 16

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agaagggttc gcacagattc cttcctggca cgctgccgtt gagcgagtta ttcctgtggc 120

aatacgtcgc tggcgtaaag cctggttctc ggcccccata aaagctgaac gcaaagcgtt 180

tcgtgaagcg ctacaagcag agaactatga cgcagttatc gacgctcagg ggctggtaaa 240

aagcgcggca ctggtgacac gtctggcgca tggcgtaaag catggattgg actggcaaac 300

cgctcgcgaa cctttagcca gcctgtttta caattgtaag catcatattg caaaacagca 360

gcacgccgta gaacgcaccc gcgaactgtt tgccaaaagt ttgggctata gcaaaccgca 420

aacccagggc gattatgcta tcgcacagca ttttctgacg aacctgccta cagatgctgg 480

cgaatatgcc gtatttcttc atgcgacgac ccgtgatgat aaacactggc cggaagaaca 540

ctggcgagaa ttgattggtt tactggctga ttcaggaata cggattaaac ttccgtgggg 600

cgcgccgcat gaggaagaac gggcgaaacg actggcggaa ggatttgctt atgttgaagt 660

attgccgaag atgagtctgg aaggcgttgc ccgcgtgctg gccggggcta aatttgtagt 720

gtcggtggat acggggttaa gccatttaac ggcggcactg gatagaccca atatcacggt 780

ttatggacca accgatccgg gattaattgg tgggtatggg aagaatcaga tggtttgtag 840

ggctccgggg aatgagttgt ctcaattgac agcaaatgct gttaagcggt tcattgaaga 900

aaacgctgcc atgatttaa 919

<210> 17
<211> 1023
<212> DNA
<213> Escherichia coli
<400> 17

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taccctgacg caaaaatcga tgtgctgctt tatcaggaca ccatcccgat cctgtctgaa 120

aatccagaga ttaacgcgct ctacggcata aaaaataaaa aagcaaaagc ctcagaaaaa 180

attgccaact ttttttcatct catcaaggta ttacgtgcca ataagtatga ccttatcgtc 240

aatctcaccg atcaatggat ggttgctata ctggttcgct tattaaatgc ccgtgtgaaa 300

atttcccagg attatcatca tcggcagtct gctttttggc gtaaaagttt cacccatttg 360

gtgccgttgc agggtggaaa tgtggtggaa agtaacttat ccgtgctgac cccattggga 420

gttgattcgt tggtgaagca gacaaccatg agttacccgc ctgcaagctg gaaacgtatg 480

cgtcgcgaac ttgatcacgc tggtgttgga caaaattatg tggttatcca acctacggcg 540

cggcaaatct tcaaatgctg ggacaacgcc aagttttccg ctgtgattga tgccttacat 600

gctcgtggtt atgaagttgt tctgacgtcc ggcccagata aagacgatct ggcctgcgtc 660

aatgaaattg cgcagggatg ccagacgcca ccagtaacgg cgctggctgg aaaggtgacc 720

ttcccggaac ttggtgcgtt aatcgatcat gcgcagctgt ttattggcgt tgattccgca 780

ccggcgcata ttgccgctgc agttaatacg ccgctgatat cgctgtttgg tgcgacagac 840

catattttct ggcgtccctg gtcaaataac atgattcaat tctgggcggg agattaccgg 900

gaaatgccaa cgcgcgatca gcgtgaccga aatgagatgt atctttcggt tattccggcg 960

gcagatgtca ttgctgctgt cgataaatta ctgccctcct ccacgacagg tacgtcgtta 1020

tga 1023

<210> 18
<211> 798
<212> DNA

<213> Escherichia coli
<400> 18

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gaaatggcgg gcaaaagcta ttttctcaaa tggcatcgcg gcacgaccct gaaagagata 180
atcaaaaatt tactctcatt gcggatgcca gtattaggcg ctgaccgcga atggaatgcg 240
attcatcgac tgcgggatgt cggcgttgat actatgtatg gggtggcatt tggcgaaaaa 300
ggcatgaatc cgctgaccag aacttcattt attattaccg aagatctgac accaaccata 360
agtctggaag attactgtgc tgactgggcg actaaccctc cagatgttcg cgtaaagcgt 420
atgcttatta agcgtgtcgc gacgatggtg cgcgatatgc atgctgcggg cattaaccac 480
cgtgactgtt atatctgtca tttcctgctg cacttgcctt tttccggtaa ggaagaggag 540
ttaaaaattt cggtaattga cctgcaccgg gcgcagcttc gcacgcgcgt tccacgtcgt 600
tggcgggata aagatcttat tgggctttat ttttcttcga tgaatatcgg cctgactcag 660
cgggatatct ggcggtttat gaaagtgtat tttgccgccc cgcttaaaga cattctcaag 720
caggaacaag gactgctgtc gcaagcagaa gcaaaagcca caaaaatcag ggaaagaacg 780
attcgaaaat cgttgtaa 798

<210> 19
<211> 1125
<212> DNA
<213> Escherichia coli
<400> 19

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atgcgtattg ctcagacagt cgccgcccga ggtcatcatg ttcgggttta tacccagtcg 120
tgggaaggcg aatgccctga tgtatttgaa ctgatcaaag tgccggttaa atcgcatacc 180
aatcacgggc gcaatgcgga gtattttgcc tgggtgcaaa aacatttacg cgaacatccc 240
gtcgataaag tcgttggatt caacaaaatg ccggggctgg acgtttatta tgccgctgat 300
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tatcgccatt atgccgcctt tgagcgggca accttcgaac agggcaagcc gacacagctg 420
ctgatgctga cagataagca aatcgccgat ttccagaaac attatcagac tgaagcggag 480
cgttttcata ttctgccacc ggggatttat cctgatcgta aatatagcca gcagccagcc 540
aatagccgtg aaatcttccg taagaagaat ggaataaccg aacaacaata tttattgttg 600
caggtcggtt cagacttcac gcgtaaaggt gtcgatcgtt ccattgaagc acttgcttcg 660
ttaccggatt cgctgcgcca caacacattg ctatatgttg ttgggcagga taaaccgcga 720
aaatttgagg cactggcaga aaaacgcggc gtgcgcagta atgttcactt cttctcgggg 780
cgcaacgatg tctcggaatt aatggcggcg gcggatttat tactgcatcc tgcctaccag 840

gaagcggcgg gaattgtgct gctggaagcg attactgcag gattaccggt actaacaact 900

gccgtttgtg gctatgcgca ttatattgtc gacgctaatt gcggcgaggc tattgctgag 960

ccattccgcc aggaaacatt gaatgagatt ttacgcaaag cgttaacgca atcttcattg 1020

cgccaggctt gggcggaaaa tgcgcgacat tatgctgata cacaagattt atacagtctg 1080

ccagagaaag cggcggatat cataacgggt ggtctggatg gttga 1125

<210> 20
<211> 1047
<212> DNA
<213> Escherichia coli
<400> 20

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tgccgtccat tattatcgcg gatgccggaa gttaacgaag ctattcctat gcctctcggt 180

cacggagcgc tggaaatcgg cgaacgccgc aaactgggtc atagcctgcg tgaaaagcgc 240

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ggtattcctc atcgcaccgg ctggcgcggc gagatgcgct acggtttact caacgatgta 360

cgcgtgctcg ataaagaagc ctggccgcta atggtggaac gctatatagc gctggcctat 420

gacaaaggca ttatgcgcac agcacaagat ctgccgcagc cattgttatg gccgcagttg 480

caggtgagcg aaggtgaaaa atcatatacc tgtaatcaat tttcgctttc atcagaacgt 540

ccgatgattg gtttttgccc gggtgcggag tttggtccgg caaaacgctg gccacactac 600

cactatgcgg agctggcaaa gcagctgatt gatgaaggtt atcaggtggt tctgtttggc 660

tcggcgaaag atcatgaagc gggcaatgag attcttgccg ctttgaatac cgagcagcag 720

gcatggtgtc ggaacctggc gggggaaaca cagcttgatc aagcggttat cctgattgca 780

gcctgtaaag ccattgtcac taacgattct ggcctgatgc atgttgcggc ggcgctcaat 840

cgtccgctgg ttgccctgta tggtccgagt agcccggact tcacaccgcc gctatcccat 900

aaagcgcgcg tgatccgttt gattaccggc tatcacaaag tgcgtaaagg tgacgctgcg 960

gagggttatc accagagctt aatcgacatt actccccagc gcgtactgga agaactcaac 1020

gcgctattgt tacaagagga agcctga 1047

<210> 21
<211> 1017
<212> DNA
<213> Escherichia coli
<400> 21

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ctttttggtt gtggtgtttc aatcacgtca gttttgttac ataacaacga cgtgagtttt 180

gttttccacg tttttattga tgatatccct gaagccgata tccagcgttt agcccaattg 240
gcgaaaagct atcgtacctg tatccagatc catctagtaa attgtgaacg gcttaaggca 300
ttaccgacga ccaaaaattg gtctattgcc atgtatttcc gttttgtaat tgcagattac 360
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ttaaagccgc tgataacaat ggatcttgcc aataacgttg ctgctgttgt tactgaacgc 480
gatgctaact ggtggtcgtt acggggtcaa agtctgcagt gtaatgaact tgaaaagggt 540
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caagatatcc ttaatcttat cctgttaggg aaagttaaat tcattgatgc taaatacaat 720
acgcaattta gtttaaatta tgaattaaaa aaatcatttg tttgtccaat taatgatgaa 780
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agtgcgcaac cttttatcaa agccaaagaa gcatcgccct ggaaaaatga accgttaatg 900
cggccagtta actcaaacta tgctcgttat tgcgccaagc ataattttaa acaaaacaaa 960
ccaattaacg ggataatgaa ttatatttat tatttttatt taaagataat aaaatga 1017

<210> 22
<211> 909
<212> DNA
<213> Escherichia coli
<400> 22

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ttaattcaat acgtcgtgaa tgaatgtatt gcggctggca ttactgaaat tgtgctggtt 180
acacactcat ctaaaaactc tattgaaaac cactttgata ccagttttga actggaagca 240
atgctggaaa aacgtgtaaa acgtcaactg cttgatgaag tgcagtctat ttgtccaccg 300
cacgtgacta ttatgcaagt tcgtcagggt ctggcgaaag gcctgggaca cgcggtattg 360
tgtgctcacc cggtagtggg tgatgaaccg gtagctgtta ttttgcctga tgttattctg 420
gatgaatatg aatccgattt gtcacaggat aacctggcag agatgatccg ccgctttgat 480
gaaacgggtc atagccagat catggttgaa ccggttgctg atgtgaccgc atatggcgtt 540
gtggattgca aaggcgttga attagcgccg ggtgaaagcg taccgatggt tggtgtggta 600
gaaaaaccga aagcggatgt tgcgccgtct aatctcgcta ttgtgggtcg ttacgtactt 660
agcgcggata tttggccgtt gctggcaaaa acccctccgg gagctggtga tgaaattcag 720
ctcaccgacg caattgatat gctgatcgaa aaagaaacgg tggaagccta tcatatgaaa 780
gggaagagcc atgactgcgg taataaatta ggttacatgc aggccttcgt tgaatacggt 840
attcgtcata acacccttgg cacggaattt aaagcctggc ttgaagaaga gatgggcatt 900
aagaagtaa 909

<210> 23
<211> 1641
<212> DNA
<213> Escherichia coli
<400> 23

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caactgacgg cgcaatatta tgtactgaaa ccagaagcag ggaatgcgga gcacgcggtg 120
aaattcggta cttccggtca ccgtggcagt gcagcgcgcc acagctttaa cgagccgcac 180
attctggcga tcgctcaggc aattgctgaa gaacgtgcga aaaacggcat cactggccct 240
tgctatgtgg gtaaagatac tcacgccctg tccgaacctg cattcatttc cgttctggaa 300
gtgctggcag cgaacggcgt tgatgtcatt gtgcaggaaa acaatggctt caccccgacg 360
cctgccgttt ccaatgccat cctggttcac aataaaaaag gtggcccgct ggcagacggt 420
atcgtgatta caccgtccca taacccgccg gaagatggtg gaatcaaata caatccgcca 480
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<210> 24

<211> 1677
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<400> 24

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<211> 624
<212> DNA
<213> Escherichia coli

<400> 25

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caatcatatc caatattagg taatgatatt gcagacatcg agaataagga taattattat 180

tattttattg ggataggcaa accatcaact aggaagcact atttaaacat cataagaaaa 240

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agattaatta gggggaatga atga 624

<210> 26
<211> 576
<212> DNA
<213> Escherichia coli
<400> 26

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<210> 27
<211> 933
<212> DNA
<213> Escherichia coli
<400> 27

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<210> 28
<211> 1434
<212> DNA
<213> Escherichia coli
<400> 28

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<211> 1263
<212> DNA
<213> Escherichia coli
<400> 29

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gcagtagcgt tgacaaaggc aggttttaaa agcagagttc aaaaagttag agctttcagt 180

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<211> 1536
<212> DNA
<213> Escherichia coli
<400> 30

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ggtgtgcaaa tttcacatga caatttattg agctttacaa attggatgat ttctgatgat 540

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<210> 31
<211> 60
<212> DNA
<213> Escherichia coli
<400> 31

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<210> 32
<211> 60
<212> DNA
<213> Escherichia coli
<400> 32

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